Claims

What is claimed is:

1. A method for the simultaneous quantitative analysis of at least three samples of molecules, comprising:

(i) reacting the molecules of each sample with a set of at least two isotopically labelled reagents, wherein each set of isotopically labelled reagents is differentially labelled, resulting in at least three differentially and isotopically labelled derivatives of molecules;

(ii) combining the derivatized molecules in a preparation for examination by mass spectrometry; and

(iii) examining the preparation by mass spectrometry.

2. A method for the quantitative analysis of a sample of molecules having an amine bearing an active hydrogen, comprising:

(i) reacting the molecules, with isotopically labelled reagents resulting in the reductive alkylation of the amines to their alkylamine derivatives, such that the alkylamine derivatives are isotopically labelled in a preparation for examination by mass spectrometry; and

(ii) examining the preparation by mass spectrometry.

3. A method for the quantitative analysis of two or more samples of molecules having an amine bearing an active hydrogen, comprising:

(i) reacting the molecules in each sample with isotopically labelled reagents resulting in the reductive alkylation of the amines to their alkylamine derivatives, such that the alkylamine derivatives are isotopically labelled;

(ii) combining the derivatized molecules in a preparation for examination by mass spectrometry; and

(iii) examining the preparation by mass spectrometry.

4. The method of claim 1, 2 or 3 comprising an additional step of cleaving the derivatized molecules in the preparation into fragments, prior to examining the preparation by mass spectrometry.

5. The method of claim 1, 2 or 3 comprising an additional step of denaturing the molecules prior to reacting the molecules with isotopically labelled reagents.

6. The method of claim 1, 2, 3 or 4 wherein the step of examining the preparation by mass spectrometry comprises introducing the preparation containing the derivatized molecules or fragments to a mass spectrometer using electrospray ionisation.

7. The method of claim 6 wherein the electrospray ionisation method is selected from a group consisting of nanospray, pneumatically assisted electrospray, ionspray and turboionspray.

8. The method of claim 1, 2 or 3 comprising an additional step of separating the derivatized molecules in the preparation before examining the preparation by mass spectrometry.

9. The method of claim 8 wherein the step of separating the derivatized molecules uses a separator selected from a group consisting of 1-D gel electrophoresis, SDS-PAGE, isoelectric focusing, 2-D gel electrophoresis, zone electrophoresis, isotachophoresis, ion exchange chromatography, normal phase chromatography, reverse phase chromatography, hydrophobic interaction chromatography, size exclusion chromatography and any combination of these separators.

10. The method of claim 4 comprising an additional step of separating the fragments after cleaving the derivatized molecules in the preparation.

11. The method of claim 10 wherein the step of separating the fragments uses a separator selected from a group consisting of liquid chromatography, high performance liquid chromatography and capillary electrophoresis.

12. The method of claims 1, 2 or 3 comprising an additional step of analyzing the preparation after examining the preparation by mass spectrometry.

13. The method of claim 12 wherein the step of analyzing the preparation is selected from a group consisting of collision-induced dissociation in a mass spectrometer

operating in MS/MS mode, peptide mass fingerprinting, peptide mapping, Edman sequencing and sequencing by sequential amino acid cleavage.

14. The method of claim 13, comprising an additional step, after the step of analyzing the preparation of sequencing the molecule.

15. The method of claim 1, 2 or 3 wherein the isotopically labelled reagents are an aldehyde and a reducing agent.

16. The method of claim 15 wherein the aldehyde is selected from a group consisting of formaldehyde and acetaldehyde.

17. The method of claim 15 wherein the reducing agent is selected from a group consisting of a sodium cyanoborohydride, sodium borohydride, dialkyl borane complexes and pyridine borane complexes.

18. The method of claim 1, 2 or 3 wherein the sample is selected from a group consisting of cells, cellular extracts, sub-cellular extracts, cellular lysates, peptides, proteins, drugs, toxins, antibodies and pollutants.

19. The method of claim 18 wherein the proteins are extracted from cells.

20. The method of claim 19 wherein the amines of the proteins are selected from a group consisting of lysine residues, ornithine residues and residues at the N-terminal amino group of the proteins.

21. The method of claim 1, 2 or 3 wherein the step of examining the preparation by mass spectrometry utilizes a mass spectrometer selected from a group consisting of:

(i) Fourier transform – Ion cyclotron resonance mass spectrometers (FT-ICR-MS);

(ii) Time of Flight mass spectrometers (TOF-MS, TOF-TOF-MS);

(iii) Ion trap mass spectrometers (IT);

(iv) Quadrupole mass spectrometers (Q-MS and QqQ-MS);

(v) Ion mobility mass spectrometers (IM-MS);

(vi) Quadrupole (or hexapole, octapole)-Time of Flight mass spectrometers (Q-TOF, and Qq-TOF); and

(vii) Ion trap – Time of flight mass spectrometers (IT-TOF).

22. The method of claim 21 comprising an additional step of combining the mass spectrometer with an ionisation source.

23 The method of claim 22 wherein the ionisation source is selected from a group consisting of electrospray ionisation, matrix-assisted laser desorption and ionisation (MALDI), field desorption, thermal desorption and laser desorption.

24. A preparation of three samples of molecules for simultaneous quantitative analysis by mass spectrometry, each sample comprising differentially and isotopically labelled derivatives of molecules, each sample resulting from a reaction of a set of at least two isotopically labelled reagents with the molecules.

25. A preparation of a sample of molecules comprising isotopically labelled derivatives resulting from the reductive alkylation of the amines to alkylamine derivatives by isotopically labelled reagents.

26. A preparation of two or more samples of molecules for the simultaneous analysis by mass spectrometry, each sample comprising differentially and isotopically labelled derivatives of molecules resulting from the reductive alkylation of the amines to alkylamine derivatives by isotopically labelled reagents.

27. Use of a mass spectrometer for the analysis of a sample according to any of claims 1, 2 or 3.

28. A kit comprising isotopically labelled reagents and instructions to follow the methods of quantitative analysis of any of claims 1, 2 or 3.

29. A method for the quantitative analysis of two or more cellular extracts comprising molecules having an amine bearing an active hydrogen, comprising:

(i) reacting the molecules of the extracts with isotopically labelled reagents resulting in the reductive alkylation of the amines to their alkylamine derivatives, such that the alkylamine derivatives are isotopically labelled;

(ii) combining the derivatized molecules of the extracts in a preparation;

(iii) separating the molecules;

(iv) enzymatically cleaving the molecules into fragments;

(v) separating the fragments;

(vi) examining the fragments by mass spectrometry; and

(vii) sequencing the fragments.